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TITLE: Development of a Mouse Model for Determination of the Role of the Catechol Metabolites of Estradiol in Mammary Tumorigenesis

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11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

The purpose of the proposed research is to develop a catechol-o-methyltransferase knockout (COMTKO)-estrogen receptor/Wnt-1 (ERKO/Wnt-1) mouse model for use in studies on the role of estrogen catechol metabolites in mammary tumorigenesis. The scope of the project involves: 1) Through genetic crossing, introduce the COMT KO genotype into the ERKO/Wnt-1 mice; 2) Initiate studies to determine the effects of the absence of COMT on estrogen catechol metabolite, glutathione-estrogen quinone adduct levels (which reflect oxidative metabolism of estrogen catechol to estrogen quinones), oxidative DNA damage levels, mammary gland development and tumorigenesis in the resulting COMTKO/ERKO/Wnt-1 female mice. ERKO/Wnt-1 mice were obtained after notification of award; COMTKO mice were on-hand. However, unanticipated difficulties were encountered. The COMTKO mice went through a period where their breeding stopped. Additional breeding pairs were obtained from the originator of the strain at Rockefeller University. These mice began breeding and at that point, the first aim could proceed, although we were already about 6 months behind given the time required to obtain additional breeding pairs, time in quarantine, and time for them to adapt and produce off-spring for the desired crosses. As detailed in the progress report, breeding is now proceeding. The project period has been extended 1 year to 20 August, 2005.

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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Table of Contents

Introduction

The main risk factors associated with the development of breast cancer implicate estradiol (E2) as a causative agent[1]. The accepted hypothesis is that prolonged exposure to estrogen leads to persistent enhanced proliferation mediated through the binding of E2 to the estrogen receptor. However, E2 also undergoes cytochrome P450 (CYP)-mediated oxidative biotransformation to catechol metabolites, 2-hydroxy (2-OH) and 4-hydroxy (4-OH) E2. 4-OH E2 in particular has been shown to cause/contribute to estrogen carcinogenesis through further oxidative metabolism to quinones that form adducts with adenine and guanine in DNA and cause oxidative DNA damage through participation in redox cycling processes[2]. These catechol estrogen metabolites are present in mouse mammary gland tissue at concentrations of pmoles/mg tissue. CEs are primarily inactivated by *O*-methylation catalyzed by catechol-*O*-methyltransferase (COMT)[1]. Inhibition of COMT enhanced E2-induced renal tumorigenesis in the Syrian hamster model[1] and DNA damage in human MCF-7 cells[3], suggesting that COMT is highly protective against adverse effects caused by the catechol metabolites of E2. COMT is polymorphic in humans and several, but not all, epidemiology studies have observed that the genotype encoding a low activity COMT is associated with an increased risk for developing breast cancer in certain women[4]. These observations implicate the catechol metabolites of E2 in breast cancer causation. Experimental investigation of the extent and mechanisms of contribution of the estrogen catechol metabolites would be greatly facilitated by the availability of an appropriate animal model. Mammary tumor incidence in intact estrogen receptor knockout (ERKO)/Wnt-1 female mice is 20%, whereas it is reduced to 8% in ovariectomized females (Richard Santen, University of Virginia, personal communication). These results demonstrate that in the absence of ER expression, estrogen still contributes to mammary tumor formation. The concept/hypothesis regarding the role of estrogen in these mice is that the catechol metabolites undergo oxidative biotransformation to quinones which then form DNA adducts directly, or oxidative DNA damage indirectly through redox cycling, and subsequently mutations, which contribute to mammary tumor formation. Since COMT is protective, the absence of COMT would be expected to increase the incidence and/or shorten the latency of tumorigenesis in the ERKO/Wnt-1 mice. Thus, the purpose of the proposed research is to develop a catechol-o-methyltransferase knockout (COMTKO)-estrogen receptor/Wnt-1 (ERKO/Wnt-1) mouse model for use in studies on the role of estrogen catechol metabolites in mammary tumorigenesis. The scope of the project involves: 1) Through genetic crossing, introduce the COMTKO genotype into the ERKO/Wnt-1 mice; 2) Initiate studies to determine the effects of the absence of COMT on estrogen catechol metabolite, glutathione-estrogen quinone adduct levels (which reflect oxidative metabolism of estrogen catechol to estrogen quinones), oxidative DNA damage levels, mammary gland development and tumorigenesis in the resulting COMTKO/ERKO/Wnt-1 female mice.

Body:

Key Accomplishments: ERKO/Wnt-1 mice were obtained after notification of award; COMTKO mice were on-hand. However, unanticipated difficulties were encountered. The COMTKO mice went through a period where their breeding stopped. Additional breeding pairs were obtained from the originator of the strain at Rockefeller University. These mice began breeding and at that point, the first aim could proceed, although we were already about 6 months behind given the time required to obtain additional breeding pairs, time in quarantine, and time for them to adapt and produce off-spring for the desired crosses. Because of these difficulties, a request was made and granted for the project to be extended to 20 August, 2005 without additional funds (see the Amendment of Solicitation/Modification of Contract document in the Appendix.

The breeding scheme has been modified to be more efficient and is shown below.

ERKO|Wnt-1—COMT Interbreeding Project

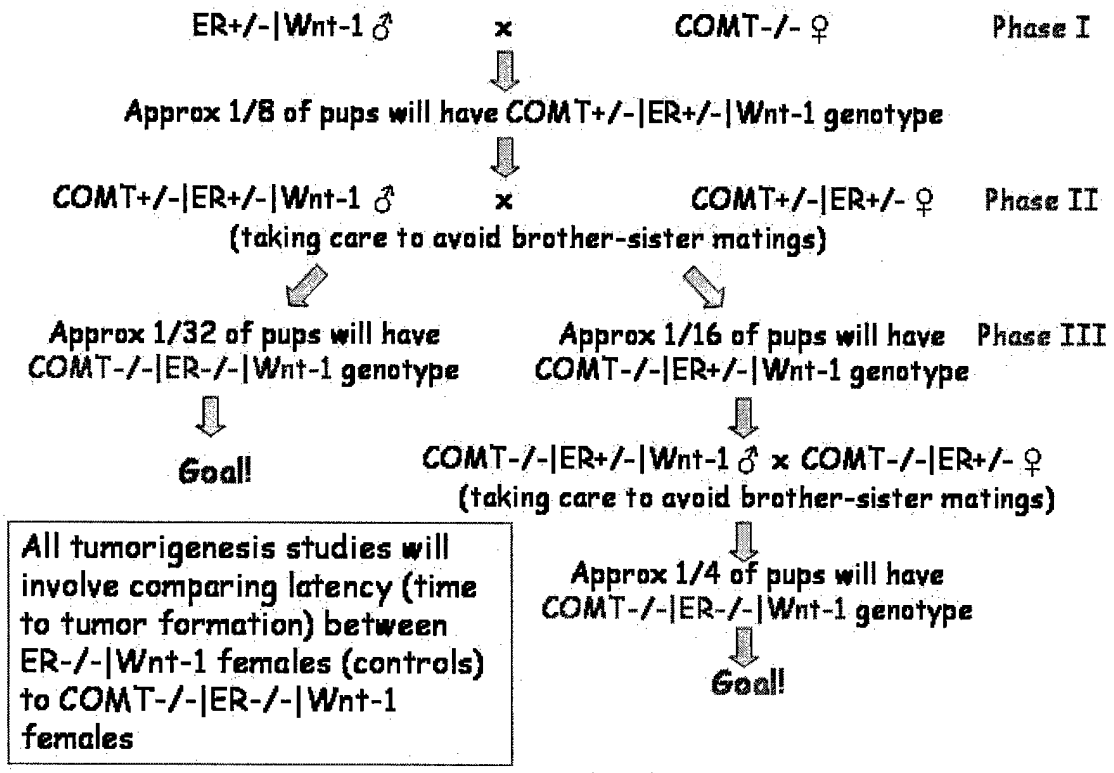

Figure 1: Breeding Scheme. Plan for creating COMT-/-|ER-/-|Wnt-1 mouse.

Phase I Breeders

Name	Male genotype	Female genotype
BPA	ER +/-|Wnt-1	ER +/+|COMT-/-
BPB	ER+/-|Wnt-1	ER+/+|COMT-/-

The PCR methods for genotyping were established in the lab. This is complex in that it involves genotyping for three genes and since both the ERKO/Wnt-1 and COMTKO mice were developed with the same selectable marker, neo, identification of the desired offspring is a bit more involved.

We obtained 32 pups from the Phase I breeding that were used in Phase II.

Phase II

We cannot directly test whether the Phase II breeders are ER+/- or ER-/- *and/or* COMT +/- or COMT-/-. This is because the same neo marker is used in both the ER-/- and the COMT-/- mice. We can only tell from their offspring. Thus far two females have been born of Phase II breeders that are negative for COMT, thus indicating that these parents in these breeding pairs are COMT+/-. The first 5 breeding pairs listed below were bred on 9/8/04, and thus far all have produced at least one litter. The last 3 breeding pairs were bred on 10/7/04, and, as of 10/27/04, have not produced litters, but it is a bit too early, and we do not expect them to produce litter for another week or two.

Phase II Breeders

Male genotype				Female genotype			
neo	Wnt	ER	COMT	neo	Wnt	ER	COMT
+	+	+	+	+	+	+ (ER+/-)	+
+	+	+	+	+	+	+(ER+/-)	+
+	+	+	+	+	+	+(ER+/-)	+
+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	-
+	+	+	+	+	-	+	+
+	+	+	+	+	-	+	+
+	+	+	+	+	-	+	+

15 Phase II pups have now been weaned and are in the Phase III stage of the breeding plan which will produce the desired ERKO/Wnt-1/COMTKO female mice.

Phase III

Several of the Phase II breeders have produced offspring, 2 of which were females negative for COMT. These two females have been bred to male offspring of Phases I and II, respectively. Their genotypes are shown below. If any of the offspring of these Phase III breeders are negative for ER *AND* COMT, we will have reached the goal of obtaining a COMT -/-| ER -/-|Wnt-1 mouse. The first Phase II breeders were put together on 10/27/04, so it will be a few weeks before we see pups.

Phase III Breeders

Male genotype				Female genotype			
neo	Wnt	ER	COMT	neo	Wnt	ER	COMT
+	+	+	+	+	-	+	-
+	+	+	+	+	-	+	-

It is important to appreciate that the breeding is a continuous process, and that as the desired mice are obtained they are used in the studies proposed.

Reportable Outcomes: None

Conclusions: The primary goal of funding provided by this grant is to allow us to produce these mice, and this is now in full swing.

References:

1. Monographs JNCI: Estrogens as endogenous carcinogens in the breast and prostate. JNCI Monographs 2000, 27:159
2. Cavalieri EL, Rogan EG: A unified mechanism in the initiation of cancer. Ann N Y Acad Sci 2002, 959:341-354
3. Lavigne JA, Goodman JE, Fonong T, Odwin S, He P, Roberts DW, Yager JD: The effects of catechol-O-methyltransferase inhibition on estrogen metabolite and oxidative DNA damage levels in estradiol-treated MCF-7 cells. Cancer Res 2001, 61:7488-7494
4. Lavigne JA, Helzlsouer KJ, Huang HY, Strickland PT, Bell DA, Selmin O, Watson MA, Hoffman S, Comstock GW, Yager JD: An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. Cancer Res 1997, 57:5493-5497
5. Devanesan P, Santen RJ, Bocchinfuso WP, Korach KS, Rogan EG, Cavalieri E: Catechol estrogen metabolites and conjugates in mammary tumors and hyperplastic tissue from estrogen receptor-alpha knock-out (ERKO)/Wnt-1 mice: implications for initiation of mammary tumors. Carcinogenesis 2001, 22:1573-1576

Appendix

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 3

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00001	28-Jul-2004	W23RYX-3137-N665	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than item 6)	CODE
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014	W81XWH	USA MED RESEARCH ACQ ACTIVITY ATTN: MARK LOHRMANN 301-619-2086 MARK.LOHRMANN@AMEDD.ARMY.MIL FORT DETRICK MD 27101	W81XWH

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		
JOHNS HOPKINS UNIVERSITY SCHOOL OF HYGIE DONNA V. HELM 615 NORTH WOLFE STREET BALTIMORE MD 21205		9A. AMENDMENT OF SOLICITATION NO.
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. DAMD17-03-1-0570
CODE 8L511 FACILITY CODE	X	10B. DATED (SEE ITEM 13) 10-Jul-2003

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

☐ C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:

X D. OTHER (Specify type of modification and authority)
Receipent's Request dated July 7, 2004

E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)

1. The purpose of this modification is to extend the period of performance for an additional 12 months in accordance with the Recipient's request dated 7 July 2004, which is incorporated herein by reference. The period of performance is changed as follows:

FROM: 21 July 2003 to 20 August 2004 (Research ends 20 July 2004)
TO: 21 July 2003 to 20 August 2005 (Research ends 20 July 2005)

2. No additional funds shall be provided for this extension period.

3. All other terms and conditions remain unchanged.

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)		16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) PATRICIA A. EVANS / CONTRACTING OFFICER TEL: 301-619-3754 EMAIL: pat.evans@us.army.mil	
15B. CONTRACTOR/OFFEROR (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY Patricia A. Evans (Signature of Contracting Officer)	16C. DATE SIGNED 29-Jul-2004

EXCEPTION TO SF 30
APPROVED BY OIRM 11-84

30-105-04

STANDARD FORM 30 (Rev. 10-83)
Prescribed by GSA
FAR (48 CFR) 53.243